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Discovery and taxonomic exploration of *Metopa propoda* sp. nov., a new species of Stenothoidae Boeck, 1871 (Amphipoda, Amphilochoidea) from a seamount of the Caroline Plate, Western Pacific

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Abstract

A new stenothoid species, *Metopa propoda* **sp. nov.**, is described from a seamount of the Western Pacific. The new species differs from all other congeneric species by the mature males having a large rectangular notch in the palmar corner of the gnathopod 2. One mitochondrial gene (COI) was used to analyze the validity of *Metopa propoda* **sp. nov.** An identification key of the Pacific *Metopa* species, including this new species, is provided.

Key Words

COI, deep sea, morphology, taxonomy

Introduction

The large amphipod family Stenothoidae Boeck, 1871 comprises 354 species and subspecies within 50 genera (Horton et al. 2024; Navarro-Mayoral et al. 2024) and is found at depths ranging from subtidal zones to over 3000 meters (Krapp-Schickel 2015; Krapp-Schickel and Vader 2015). This family is regarded as one of the most challenging among all amphipod families (Tandberg 2011) and is characterized by a vestigial coxa 1 that is covered by following coxae, a shield-like coxa 4 that is enlarged and not posterodorsally excavate, and a uniramous uropod 3 (Barnard and Karaman 1991). Among all stenothoid genera, the genus *Metopa* Boeck, 1871, has long been considered polyphyletic (Barnard and Karaman 1991; Krapp-Schickel and Koenemann 2006; Tandberg 2011; Tandberg and Vader 2023).

Following *Stenothoe* Dana, 1852, the *Metopa* is the second largest genus in the family Stenothoidae, encompassing 56 accepted species (Krapp-Schickel 2009; Tandberg 2011; Tandberg and Vader 2023; Horton et al. 2024). Krapp-Schickel and Vader (2015) categorized *Metopa* into three groups based on the shape of the palmar corner of gnathopod 1, noting that the palmar shape of gnathopod 2 is not meaningful for cladistic analysis (Tandberg 2011; Krapp-Schickel and Vader 2015). Fifteen *Metopa* species have been reported from the Western Pacific, occurring at depths up to 2300 meters (Pirlot 1933; Gurjanova 1948, 1952, 1955; Krapp-Schickel 2009). Among these, only *M. abyssi* Pirlot, 1933, and *M. torbeni* Krapp-Schickel, 2009, have been found in the tropical Western Pacific, at depths of 100 m and 54 m, respectively (Pirlot 1933; Krapp-Schickel 2009).

During a biodiversity survey of seamounts in the Caroline Plate, several stenothoid specimens were collected. After careful examination, these specimens were identified as belonging to the genus *Metopa*. The present study describes a new *Metopa* species, highlights the

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morphological distinctions between the new species and other congeneric species, and provides an identification key to all Pacific species of the genus.

Materials and methods

Specimens were collected from seamounts in the Caroline Plate of the Western Pacific by the research vessel KEXUE with Remote Operated Vehicle (ROV) FAXIAN. The animals were sorted on board and fixed in 96% ethanol, then transferred to 75% ethanol in the laboratory for further study. All material is deposited at the Marine Biological Museum of Chinese Academy of Sciences (MBMCAS), Qingdao, China. External morphology and internal anatomy were examined, dissected, and photographed under a microscope (ZEISS Discovery V20). Line drawings were completed by the software Adobe Photoshop CS6 with a graphics tablet (Wacom PTH 851). Length measurement was made along the outline of the animal, beginning from the anterior margin of the head to the posterior margin of the telson.

Genomic DNA of *Metopa propoda* sp. nov. was obtained from one specimen using Illumina HiSeq sequencing. At least 3 μg of genomic DNA was used for sequencing library construction. Paired-end libraries were prepared following Illumina's standard genomic DNA library preparation procedure (insert size of ~400 bp). Purified genomic DNA was sheared into smaller fragments with a desired size by Covaris, and blunt ends were generated by using T4 DNA polymerase. After adding an "A" base to the 3' end of the blunt phosphorylated DNA fragments, adapters were ligated to the ends of the DNA fragments. The desired fragments were purified through gel electrophoresis and then selectively enriched and amplified by PCR. The index tag was introduced into the adapter at the PCR stage, as appropriate, and we did a library quality test. Finally, the qualified Illumina paired-end library was used for Illumina Nova-Seq 6000 sequencing (150 bp*2, Shanghai Biozeron Co., Ltd). The raw paired-end reads were trimmed and quality controlled by Trimmomatic with parameters (SLIDINGWINDOW:4:15 MINLEN:75) (v. 0.36 http://www.usadellab.org/cms/uploads/supplementary/Trimmomatic). Clean data obtained by the above quality control processes were used to do further analysis. One gene (COI, 1534 bp) was BLASTed and deposited in GenBank (for accession numbers, see Table 1).

The sequences obtained were aligned using MEGA 6 (Tamura et al. 2013). The phylogenetic tree was constructed with maximum likelihood (ML), and the ML analyses were conducted online using W-IQ-TREE (http://iqtree.cibiv.univie.ac.at/) (Trifinopoulos et al. 2016), with clade support evaluated via 10,000 ML bootstrap replications. A total of fifty described and undescribed species within Stenothoidae and two outgroup taxa of Iphimediidae Boeck, 1871, were used for assessing the hypothesis that the specimen is a distinct new species by multiple species delimitation methods. The Bayesian implementation of the Poisson Tree Processes (bPTP) species delimitation model was employed as per Zhang et al. (2013), conducted on the web server of the Heidelberg Institute for Theoretical Studies, Germany (http://species.h-its.org/), using BI phylogenetic trees as the input data. The alignment from the fast-evolving COI gene was uploaded to the online server of Assemble Species by Automatic Partitioning (ASAP) method (https://bioinfo.mnhn.fr/abi/public/asap) with the model of Jukes-Cantor (JC69) with default settings (Puillandre et al. 2021). The Automated Barcode Gap Discovery (ABGD) analysis using a web-based interface (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) as described by Puillandre et al. (2012). The analysis was conducted using the Kimura 2-parameter substitution model (TS/TV = 2.0), with a prior range for maximum intraspecific divergence set between 0.001 and 0.1, encompassing 10 recursive steps, and a relative gap width (X) of 1.0.

Systematic account

Order Amphipoda Latreille, 1816
Suborder Amphilochidea Boeck, 1871
Superfamily Amphilochoidea Boeck, 1871
Family Stenothoidae Boeck, 1871
Subfamily Stenothoinae Boeck, 1871
Genus *Metopa* Boeck, 1871

***Metopa propoda* sp. nov.**

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Figs 1–3

Material examined. ***Holotype.*** Western Pacific • 1 male, 6.1 mm; an unnamed seamount in Caroline Plate; M6091, 10°04'N, 140°9–15'E; depth 985–2016 m; 2–11 Jun. 2019; collected by a team of ROV "FAXIAN"; MBM 286611.

Paratype. Western Pacific • 1 male, 4.3 mm; an unnamed seamount in Caroline Plate; M6091, 10°04'N, 140°9–15'E; depth 985–2016 m; 2–11 Jun. 2019; collected by a team of ROV "FAXIAN"; MBM 286611; 9 females, 2.3–4.5 mm; an unnamed seamount in Caroline Plate; M6091, 10°04'N, 140°9–15'E; depth 985–2016 m; 2–11 Jun. 2019; collected by a team of ROV "FAXIAN"; MBM 286821.

Description. ***Head.*** Head length subequal to pereonite 1 and 2 combined. Interantennal lobe strongly projecting, broadly rounded. Eyes rounded. Antenna 1 and 2 sexual dimorphisms. In female antenna 1, subequal to or slightly longer than antenna 2, peduncular articles 1 and 2 of equal length, flagellum 12–13 articles, accessory flagellum absent; antenna 2 peduncular article 5 the longest, flagellum 3/4 length of peduncular article 5, with 7–8 articles. In males, antenna 1 is shorter than antenna 2, primary flagellum article 1 longest, article 2 half-length of article 1 (based on male paratype); antenna 2 with flagellum shorter than half-length of peduncular article 5.

Table 1. Details of species and GenBank accession numbers used in this study.

Genus	Species	voucher/isolate	COI
Metopa Boeck, 1871	*M. propoda* **sp. nov.**	MBM 286611	PQ287283
	Metopa sp.	NUN-0300	MG320561
	M. boeckii	08PROBE-0337	MG319126
	M. boeckii	DZMB-HH 54716 c	MG264880
	M. boeckii	DZMB-HH 54693	MG264843
	M. boeckii	DZMB-HH 54688	MG264763
	M. cistella	BCAMP0079	MG310759
	M. alderi	MT03961	KT209338
	M. alderi	MT03958	KT208542
	M. rubrovittata	MT03932	KT209277
	M. rubrovittata	MT03929	KT209052
	M. rubrovittata	MT03931	KT208825
	M. rubrovittata	MT03930	KT208695
Parametopella Gurjanova, 1938	*P. cypris*	SERCINVERT2582	OQ323416
	P. cypris	SERCINVERT2541	OQ323322
	P. cypris	SERCINVERT2544	OQ323121
	P. cypris	SERCINVERT2543	OQ322893
	P. cypris	SERCINVERT2602	OQ322697
Stenothoe Dana, 1852	*S. nhatrangensis*	B	MH128324
	S. nhatrangensis	A	MH128323
	S. valida	SERCINVERT2483	OQ322684
	Stenothoe sp.	M3.Oa-3-Ssp	OQ417149
	S. gallensis	ABC-9	OL311534
	S. gallensis	ABC-5	OL311530
	S. gallensis	ABC-14	OL311539
	Stenothoe sp.	M1.c-89-Sts	MZ504220
	Stenothoe sp.	PD008SHx1	MT317094
	Stenothoe sp.	PD034GA2	MT317092
	Stenothoe sp.	PD034Gx1	MT317091
	S. marina	Crust 18897V	MG934996
	S. marina	MT02094	KT209198
	S. marina	MT02093	KT208537
	S. monoculoides	MT03107	KT209271
	S. monoculoides	MT03925	KT209192
	S. monoculoides	MT03934	KT208458
	S. monoculoides	MT03935	KT208446
Stenula Barnard, 1962	*Stenula* sp.	SFCM9-003	HM422231
	Stenula sp.	SFCM9-001	HM422230
Wollastenothoe	*W. minuta*	nl2	PP595991
	Stenothoidae sp.	BCC2010-045	MG321169
	Stenothoidae sp.	NUN-0004	MG320587
	Stenothoidae sp.	NUN-0003	MG317721
	Stenothoidae sp.	CCNUN604	MG317164
	Stenothoidae sp.	NUN-0139	MG313209
	Stenothoidae sp.	NUN-0066	MG311622
	Stenothoidae sp.	BIOUG<CAN>:BCC2010-046	HQ945467
	Stenothoidae sp.	BMBM-0990	MH242980
	Stenothoidae sp.	BMBM-0994	MH242979
	Stenothoidae sp.	BMBM-0998	MH242981
	Stenothoidae sp.	31	EF989710
Iphimedia Rathke, 1843	*I. obesa*	Crust 17980V	MG935235
	I. obesa	Crust 17979V	MG935137

Mouthparts. Right mandible incisor with four large teeth; lacinia mobilis serrate edge broader than that of the incisor, with 9 teeth; palp 3-articulate, basal article quadrangular, second one the longest, very short and small third article carrying one long distal seta. Left mandible incisor with 11 teeth unequal in size, lacinia mobilis absent. Maxilla 1 palp with one article, with row of 4 robust setae in distal 1/4 length of medial margin and 3 setae subapically. Maxilla 2 plates in ordinary tandem position. Maxilliped inner plate fused with apical notch and small marginal setae; outer plate visible as acute tooth-shaped prolongation; dactylus subequal in length to article 3.

Coxa 2 tongue-shaped, with marginal setae; coxa 3 larger than coxa 2, rectangular; coxa 4 not excavated, about 1.3 times wider than long.

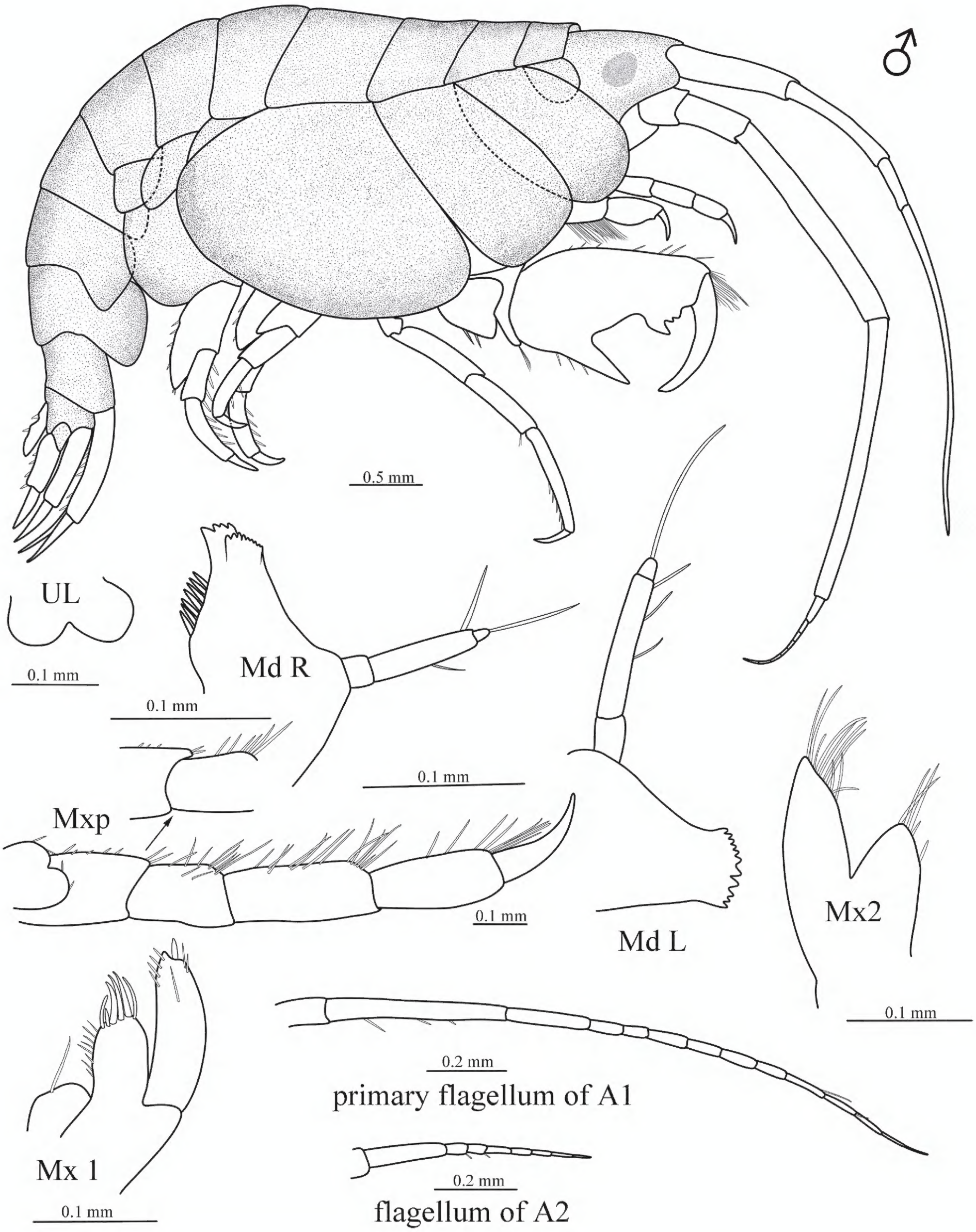

Figure 1. *Metopa propoda* sp. nov. male holotype (MBM 286611). UL. upper lip; LL. lower lip; Md L. left mandible (not showing molar and palp); Md R. right mandible; Mx1. maxilla 1; Mx2. maxilla 2; Mxp. right maxilliped; A1. antenna 1; A2. antenna 2.

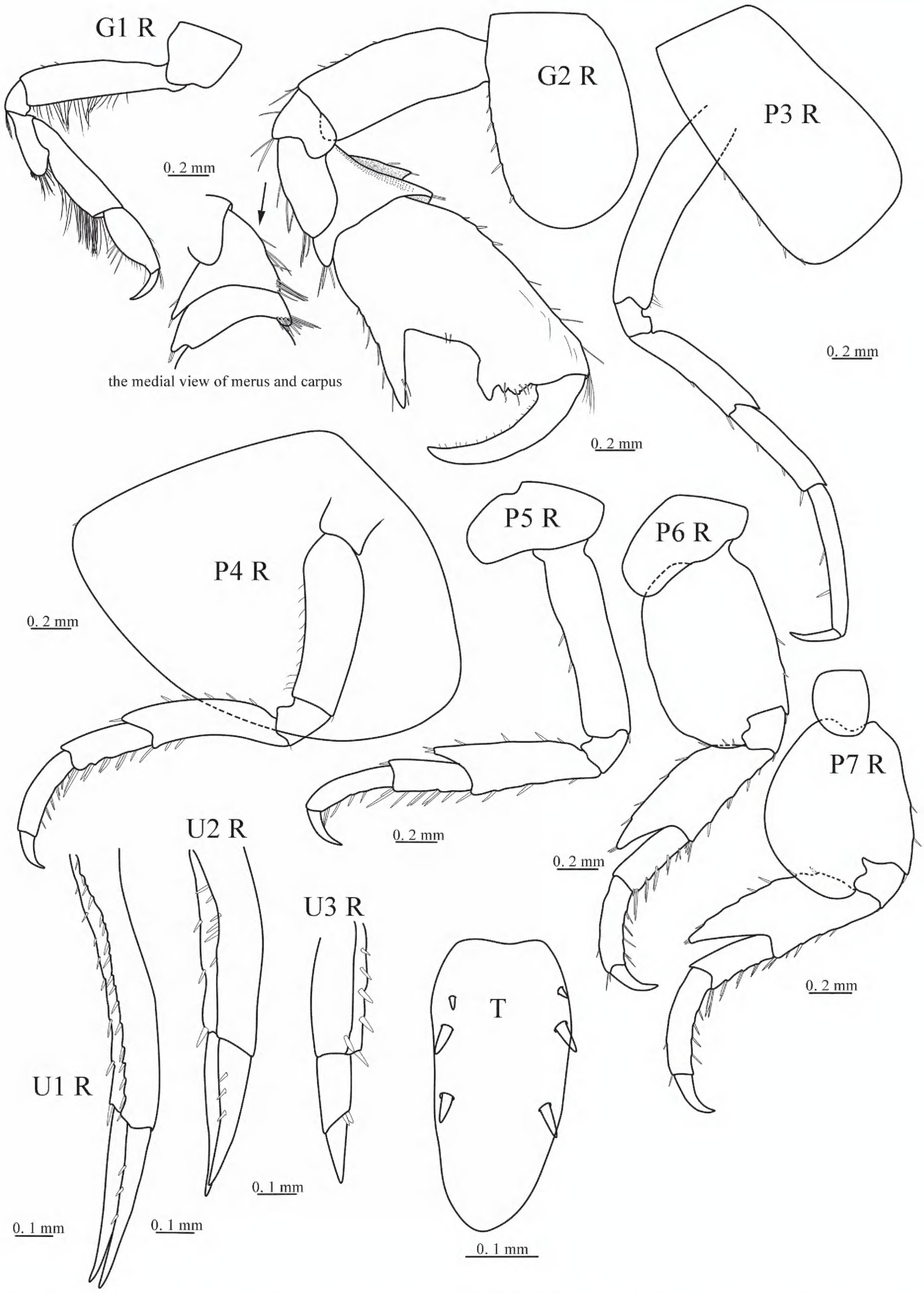

Figure 2. *Metopa propoda* sp. nov. male holotype (MBM 286611). G1 R. right gnathopod 1; G2 R. right gnathopod 2 (arrow showing the medial view of merus and carpus); P3 R. right pereopod 3; P4 R. right pereopod 4; P5 R. right pereopod 5; P6 R. right pereopod 6; P7 R. right pereopod 7; U1 R. right uropod 1; U2 R. right uropod 2; U3 R. right uropod 3; T. telson.

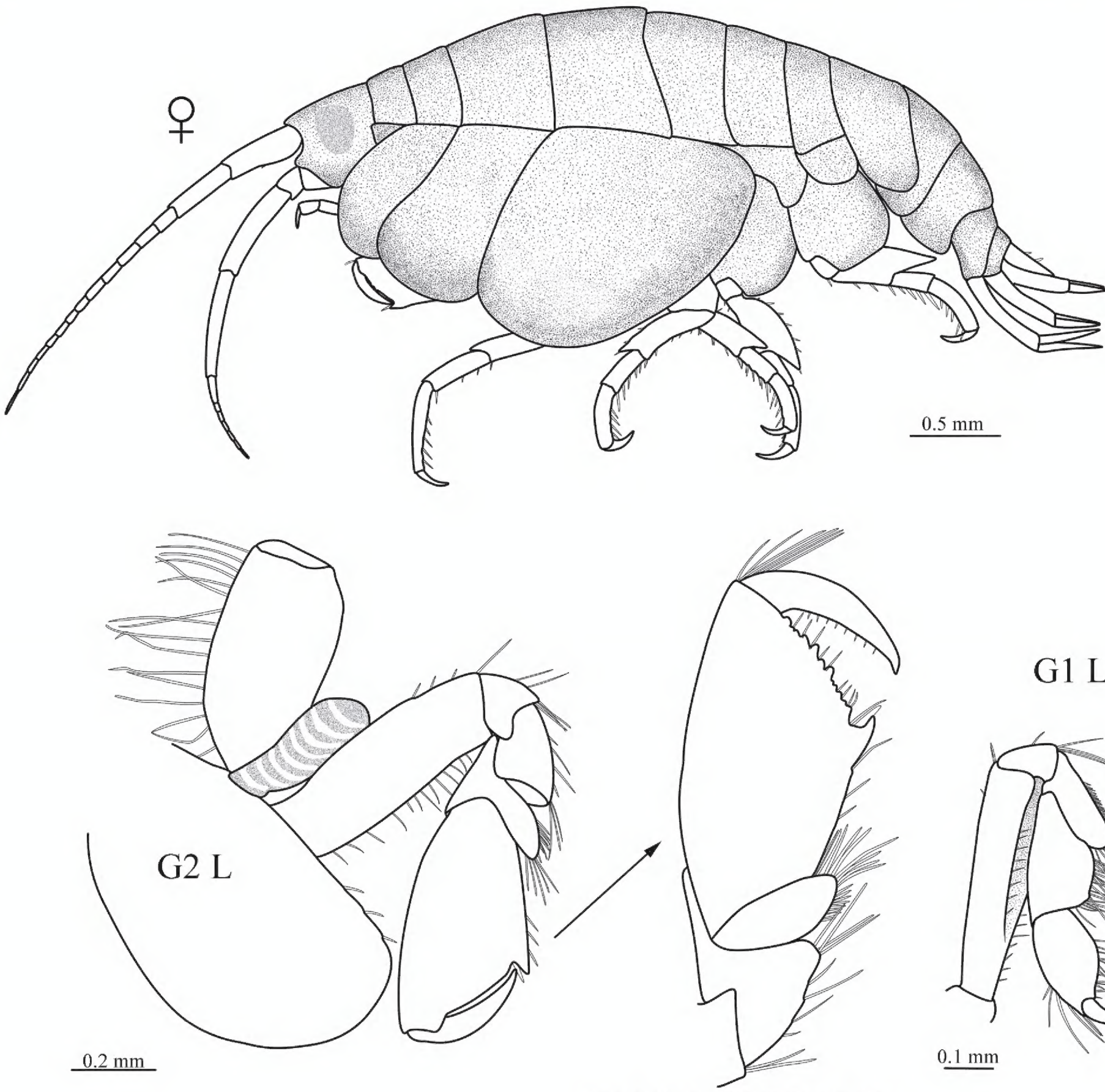

Figure 3. *Metopa propoda* sp. nov. female paratype (MBM 286821). G1 R. right gnathopod 1; G2 R. right gnathopod 2 (arrow showing the medial view of merus to dactylus).

Gnathopods and pereopods. Gnathopod 1 sexual dimorphism, propodus suboval and narrow, palm not defined; carpus in female wider and shorter than that of in male, longer than propodus, proximally narrower than distally; merus projecting under carpus, all articles beset with groups of short setae. Gnathopod 2 sexual dimorphism, female propodus slightly widening distally, hind margin subequal in length to palm, which is defined by a shallow notch and not very strong tooth and has many small serrations next to dactylus insertion; dactylus somewhat shorter than length of palm; carpus in lateral view wider than long, triangular, in medial view oval-shaped; merus not lobate, in lateral view rectangular, in medial view triangular. Gnathopod 2 male propodus hind margin shorter than palm, which is defined by a very large rectangular excavation and strong thumb-shaped palmar corner and has 4 small serrations; carpus in lateral view triangular, dorsal margin with row of stridulating knobs, in medial view rectangular, with anterodistal acute projection; merus not lobate. Pereopod 3 longer than pereopod 4, all articles elongate and weak, distal three articles with setae on posterior margin. Pereopod 4 with posterior margin of merus, carpus, and propodus with a few transverse rows of small setae; merus somewhat curved. Pereopod 5 basis rectilinear; merus to propodus anterior margin with several transverse rows of small setae; merus wider than carpus, with shortly lengthened posterodistally. Pereopod 6 and 7 bases widened with rounded posterodistal lobe; merus with lengthened posterodistally hardly reaching to distal margin of carpus; merus to propodus of both armed as in pereopod 5.

Uropods and telson. Uropod 1 peduncle much longer than subequal rami, with short robust setae on peduncle and outer ramus; inner ramus unarmed with setae. Uropod 2 peduncle longer than rami, with short robust setae; outer

ramus slightly longer than inner one, armed with robust setae; inner ramus unarmed. Uropod 3 peduncle subequal to ramus; article 1 of ramus slightly longer than spine-shaped robust article 2. Telson tongue-shaped, distally rounded, with three marginal robust setae on each side dorsally.

Etymology. The species is named for the large rectangular notch on the palmar corner of gnathopod 2.

Distribution. Presently known only from a seamount of the Caroline Plate at a depth of 985–2016 meters.

Remarks. According to the diagnostic key by Barnard and Karaman (1991), *M. propoda* sp. nov. definitely belongs to the genus *Metopa* for having the basis of pereopods 6 and 7 similarly expanded, the palp of maxilla 1 only 1-articulate, and the mandibular palp 3-articulate. Krapp-Schickel and Vader (2015) classified members of the genus *Metopa* into three groups based on the shape of the palmar corner on the gnathopod 1. *M. propoda* sp. nov. belongs to the N group, characterized by a palmar corner angle of 150–160°. Therefore, the present new species is in the similar group to the following four Pacific species: *M. abyssi* Pirlot, 1933; *M. exigua* Krapp-Schickel, 2009; *M. layi* Gurjanova, 1948; and *M. uschakovi* Gurjanova, 1948 (Krapp-Schickel 2009; Krapp-Schickel and Vader 2015).

Metopa propoda sp. nov. can be distinguished from these four species by the large rectangular notch in the palmar corner of the gnathopod 2 in mature males. Additionally, the new species differs from *M. abyssi* in having antenna 1 that is shorter or equal in length to antenna 2, rather than longer; the inner plate of the maxilliped is fused; and the merus prolongation of pereopods 5–7 does not reach the distal margin of the carpus (Pirlot 1933, fig. 54). It differs from *M. exigua* by having antenna 1 shorter or equal in length to antenna 2, a fused inner plate of the maxilliped, the merus prolongation of pereopods 6 and 7 nearly reaching the distal margin of the carpus, and the rami of uropod 2 being equal in length (Krapp-Schickel 2009). It differs from *M. layi* in that the peduncle of uropod 3 is equal in length to the ramus, whereas in *M. layi*, the peduncle is distinctly shorter than the ramus (Gurjanova 1948, fig. 49). It differs from *M. uschakovi* in that the articles of the ramus of uropod 3 are equal in length, whereas in *M. uschakovi*, article 1 is much shorter than article 2 (Gurjanova 1948, fig. 55). A key (modified from Krapp-Schickel and Vader 2015) to the Pacific species of *Metopa* is provided below.

The phylogenetic trees produced by ML analyses (Fig. 4) show that *Metopa* species cluster together with four unidentified Stenothoidae species and *Wollastenothoe minuta* Gouillieux & Navarro-Mayoral, 2024, with moderate support (BP = 66%). The *Stenothoe* species form a clade with one unidentified *Stenula* species and *Parametopella cypris* Holmes, 1905, also with moderate support (BP = 85%). The remaining two unidentified *Stenothoe* J.L. Barnard, 1962 species. and one Stenothoidae species are grouped into another moderately supported clade (BP = 87%). The species delimitation based on ABGD, ASAP, and bPTP methods has confirmed the identification of the new species (Fig. 5).

Identification key to the Pacific species of *Metopa*

1	Gnathopod 1 with palmar corner 120°, propodus widened	2
–	Gnathopod 1 simple, with palmar corner absent, propodus hind margin straight	10
–	Gnathopod 1 normal, palmar corner 150–160°, propodus hind margin rounded	14
2	Hind margin of propodus of gnathopod 2 longer than palm	3
–	Hind margin of propodus of gnathopod 2 shorter than or subequal to palm	5
3	Carpus of gnathopod 2 carrying acute, triangular lobe posterodistally	*M. majuscula* Gurjanova, 1948
–	Carpus of gnathopod 2 normal, not lobate	4
4	Article 2 of ramus of uropod 3 longer than article 1	*M. timonovi* Gurjanova, 1955
–	Article 2 of ramus of uropod 3 shorter than article 1	*M. colliei* Gurjanova, 1948
5	Merus of gnathopod 2 carrying large lobe anterodistally`	*M. mirifica* Gurjanova, 1952
–	Merus of gnathopod 2 not lobate	6
6	Body carinate	*M. eupraxiae* Krapp-Schickel, 2009
–	Body not carinate (not sure for *M. kobjakovae*)	7
7	Telson richly spinose	*M. kobjakovae* Gurjanova, 1955
–	Telson with at most several marginal robust setae on each side	8
8	Eyes absent; antenna 1 longer than antenna 2	*M. samsiluna* J.L. Barnard, 1966
–	Eyes present; antenna 1 shorter than antenna 2	9
9	Dactylus of gnathopod 2 shorter than palm	*M. japonica* Gurjanova, 1952
–	Dactylus of gnathopod 2 equal to palm	*M. bulychevae* Gurjanova, 1955
10	All peduncular articles of antenna 1 subequal in length	*M. angustimana* Gurjanova, 1948
–	Basal two peduncular articles of antenna 1 much longer than article 3	11
11	Palmar corner of gnathopod 2 not defined	*M. torbeni* Krapp-Schickel, 2009
–	Palmar corner of gnathopod 2 defined by a pronounced tooth	12
12	Carpus of gnathopod 1 subequal in length to propodus	*M. cistella* Barnard, 1969
–	Carpus of gnathopod 1 longer than propodus	13

13 Palm of gnathopod 2 nearly smooth ..... *M. dawsoni* J.L. Barnard, 1962
– Palm of gnathopod 2 carrying large tooth ..... *M. koreana* Gurjanova, 1952
14 Peduncle of uropod 3 shorter than ramus 15
– Peduncle of uropod 3 subequal to ramus 17
15 Article 1 of ramus of uropod 3 shorter than article 2 ..... *M. uschakovi* Gurjanova, 1948
– Articles of ramus of uropod 3 subequal in length 16
16 Inner plate of maxilliped not fused ..... *M. abyssi* Pirlot, 1933
– Inner plate of maxilliped fused ..... *M. layi* Gurjanova, 1948
17 Antenna 1 shorter than or subequal to antenna 2 ..... *M. propoda* sp. nov.
– Antenna 1 longer than antenna 2 ..... *M. exigua* Krapp-Schickel, 2009

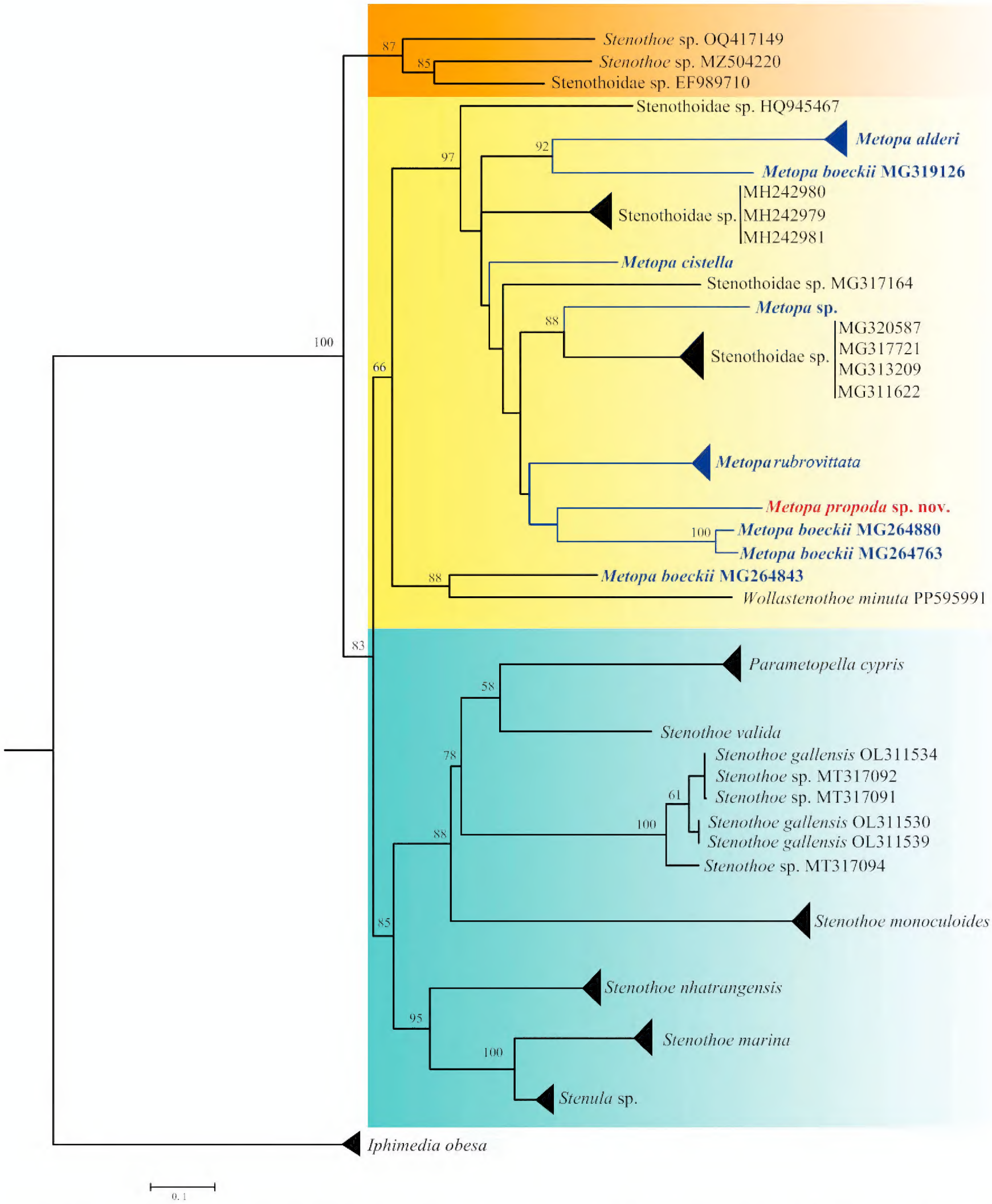

Figure 4. The maximum-likelihood (ML) tree shows the relationships between *Metopa propoda* sp. nov. and other Stenothoidae COI sequences. The numbers at each node represent bootstrap values (BP). *M. propoda* sp. nov. is highlighted in bold and red, and the other *Metopa* species are highlighted in bold and blue.

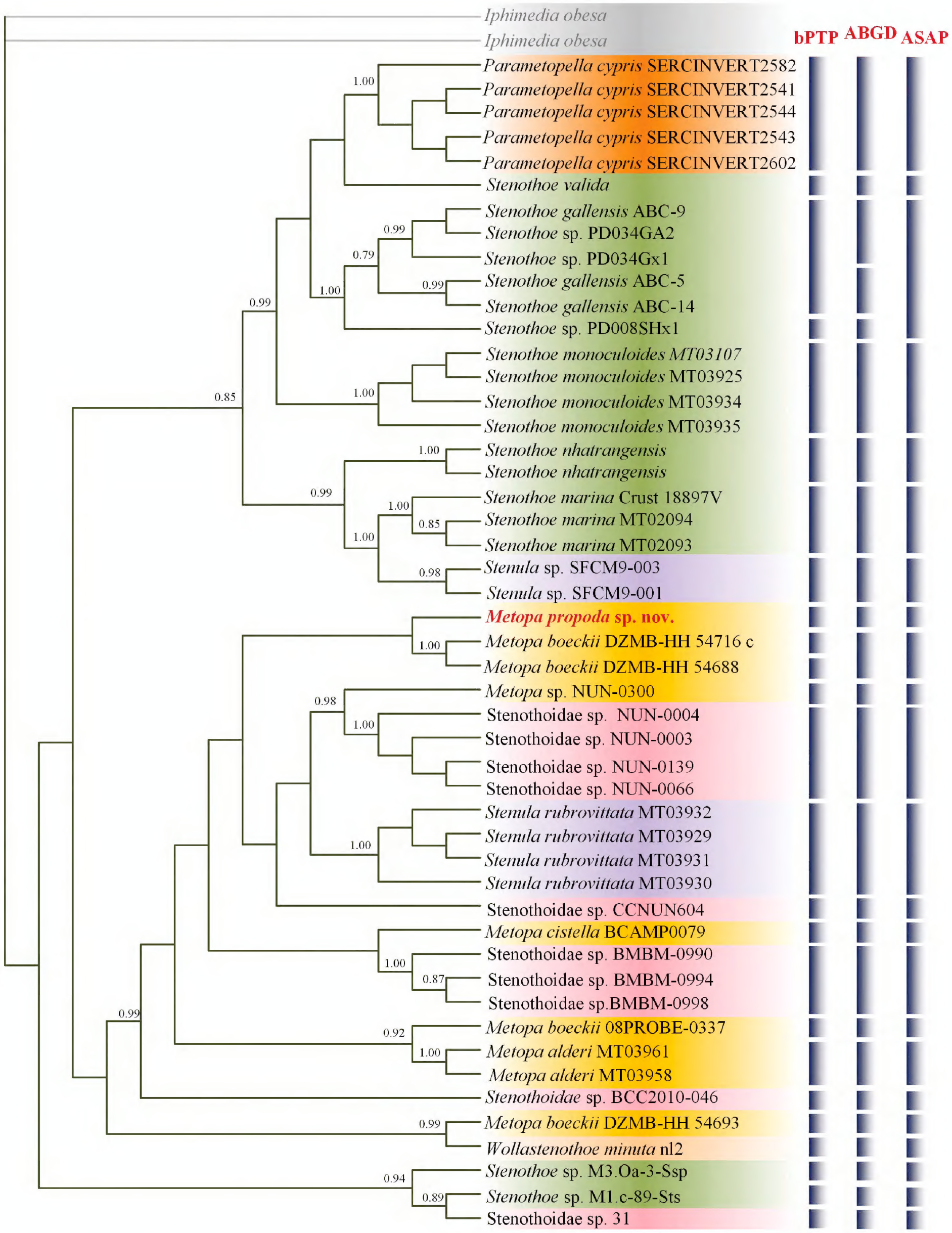

Figure 5. Bayesian inference (BI) Phylogenetic tree based on COI showing the phylogenetic relationship between *Metopa propoda* sp. nov. and related stenothoids. Numbers adjacent to nodes refer to BI posterior probability (>70). Putative species identified by DNA-based species delimitation methods [Bayesian implementation of the Poisson Tree Processes (bPTP), Automated Barcode Gap Discovery (ABGD), and Assemble Species by Automatic Partitioning (ASAP)] applied on the COI tree/distance matrices are indicated by bars on the concatenated tree.

Discussion

Nineteen *Metopa* species, including *M. propoda* sp. nov., have been reported in the Pacific, with all of them found in the northern Pacific (Gurjanova 1948, 1952, 1955; Barnard 1962, 1966, 1969; Krapp-Schickel 2009; Krapp-Schickel and Vader 2015). Five of these species are found in deep waters, including *M. angustimana* Gurjanova, 1948 (Peter the Great Gulf, 351 m), *M. bulychevae* Gurjanova, 1955 (Japan Sea, 414 m), *M. koreana* Gurjanova, 1952 (off Korea, 1100 m), *M. mirifica* Gurjanova, 1952 (Kuril Islands, 2300 m), and *M. propoda* sp. nov. (Seamount in Caroline Plate, 985–2016 m) (Gurjanova 1948, 1952, 1955). However, only the present new species was found in the deep waters of the tropical Pacific (4–5 °C).

The results of the present study validate *M. propoda* sp. nov. and reveal a close relationship between the new species and *M. boeckii* G.O. Sars, 1892, given the existing Stenothoidae molecular sequences. *Metopa boeckii* was redescribed by Tandberg (2010) and was distributed from the Arctic to the Atlantic at depths of 10–170 m. The present study reveals that *M. boeckii* showed intraspecific variation considerably exceeding the values commonly used for amphipod species delimitation (Fig. 5), indicating potential cryptic diversity (Jażdżewska et al. 2018) or misidentification, as *M. boeckii* had been confused with *M. borealis* G.O. Sars, 1883, and *M. bruzelii* (Goës, 1866) (Tandberg 2010).

The genus *Metopa* has long been considered polyphyletic (Barnard and Karaman 1991). Phylogenetic studies based on both morphological characters and molecular sequences have shown that it is not monophyletic (Krapp-Schickel and Koenemann 2006; Tandberg 2011; Navarro-Mayoral et al. 2024). Morphologically, *Metopa* has a close relationship with *Metopoides* Della Valle, 1893; *Proboloides* Della Valle, 1893; *Prometopa* Schellenberg, 1926; and *Stenothoe* Dana, 1852 (Barnard and Karaman 1991). However, the genus *Metopa* does not show a close relationship with *Stenothoe* species in the present study (Fig. 4) and Navarro-Mayoral et al. (2024). The Stenothoidae shows to be a controversial amphipod family, calling for further taxonomic and phylogenetic studies.

Acknowledgements

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